REMARKS

This amendment is presented in the new proposed format with ~~strikethrought~~ for deletions and each section of the amendment starting on a separate page. The non-elected claims have been cancelled in the amendment.

Claims 1-10, 13-18, 21-29, 32-36, 44, 45, 48-68 and 75-78 are pending. Claims 2, 3, 9, 10, and 60 are amended herein.

Rejections under 35 U.S.C. 112, first paragraph.

In the Office Action the Examiner rejected Claims 1-10, 13-18, 21-29, 32-36, 44-45, 48-68 and 75-78 under 35 U.S.C. 112, first paragraph as containing subject matter not described in the specification in such a way as to enable on skilled in the art to make and/or use the invention. The Examiner questioned the apparent lack a statistically significant association in the data. The Examiner also questioned whether the described method was of predictive value and whether it provided a guide to classification. Finally the Examiner pointed out that while the claims are drawn to detecting any malignant tumor by determining overexpression of laminin α4, the presented examples are based on brain tumors. The Examiner's point being that tumors other than brain tumors are not enabled.

Applicants respectfully traverse these rejections and will address each point made by the Examiner. The inventive method is based on the discovery that of the plethora of genes up-regulated and down-regulated in a variety of malignancies, one up-regulated gene common to a range of malignant tissues (and hitherto not known to be associated with malignancy) is the gene for the

laminin α4 subunit. Further, Applicants demonstrate that malignant and pre-malignant tissues actually express the laminin α4 peptide. The introduction explains that this particular peptide is associated with certain embryonic tissues and is found at relatively low levels in a number of normal tissues. The highest levels in normal tissues are found in intestine, skeletal muscle, liver, lung and ovary. However, only weak expression has been observed in pancreas, testis, prostate, spleen, kidney and brain (page 6 of specification). It is believed that this protein may be associated with the vasculature that malignant tissues induce to feed their growth. It is also possible that this type of laminin facilitates the invasion and metastasis of the malignancy. The present invention is based on the discovery of the association of laminin α4 with malignancy and the creation of a technique to create normal profiles of the various tissues so that increased expression of either the laminin α4 gene (mRNA) or the actual peptide can be readily detected.

On pages 3-4 of the Office Action the Examiner poses a number of questions about relative levels of expression that suggest that the Examiner may have lost the thread of the discussion in the specification. For example, the Examiner states that there is no "statistically significant association" because the analysis is "qualitative". Applicants respectfully traverse these statements. The present study is quantitative and highly statistically significant. In the case of the gene array analyses of brain malignancies, the first step was to compare normal brain tissue with an aggregate control of corpus callosum. As explained in the specification, corpus callosum is primarily composed of glial cells and forms a good control for the cell type from which the studied brain malignancies arise. It is also explained in the specification that ethical reasons make it impossible to obtain truly normal control tissue

from a patient. Clearly, it would be unethical to damage a portion of the brain in the hemisphere opposite the malignancy (this would also require additional openings be cut in the patient's skull). The present study demonstrates that samples of brain tissue taken near the malignancy are not in fact normal and cannot serve as a true control. Therefore, normal brain tissue from trauma victims was compared to the readily available corpus callosum collection. Fig 1A shows that normal brain (on one axis) compared to corpus callosum (on the other axis) produces essentially a straight diagonal collection of points almost entirely enclosed within the ±2 bars. This shows a high degree of similarity and correlation between normal brain from a patient and the corpus callosum collection. This qualifies the corpus callosum collection as an effective surrogate control for the obviously malignant tissues and for the tissues surrounding the malignancies that are demonstrated by this method to be abnormal. Figures 1B, C and D show the various malignant and abnormal tissues compared to the corpus callosum collection as control. The large number of points falling beyond the ±2 limits indicates genes whose expression differs strongly between the control and the test tissue. Note that these measurements are quantitative, reproducible and allow statistical analyses. Statistical significance is mentioned at many points in the specification, but the Examiner's attention is particularly drawn to pages 50-52 in the specification. Contrary to the contentions of the Examiner, the normal ranges are provided (the ±2 lines in the histograms) and valid statistical analyses are given.

Applicants wish to briefly discuss the difficulties the Examiner had with Table 7. This table shows the distribution of various laminin chains (as determined by immunofluorescence) in various abnormal tissues. The point

here is to show that the abnormal expression of α4 laminin mRNA as determined by gene array actually leads to detectable α4 laminin expression. Applicants believe that the Examiner may be becoming confused by trying to compare "normal" tissue from patients with any other samples. As explained in the specification and alluded to above, the tissue excised within a few centimeters of a malignancy is very definitely not "normal". Although the tissue appears normal in terms of cytology, the method of the present invention shows abnormal expression of a number of genes. The level of abnormality in expression correlates with reoccurrence of the tumor. If the adjacent tissues show expression very similar to the original tumor, reoccurrence occurs very rapidly. It is not know if the abnormal expression is due to the presence of invading tumor cells that are missed in the cytological examination or is due to influences of factors diffusing from the original tumor. Applicants believe that the statement quoted by the Examiner: "All GBMs and their adjacent tissues highly expressed laminin α4 subunit gene. Meningioma from patient 38 and normal brain from patient 46 had lower levels of α4 laminin subunit gene expression than glial tumors, but higher than normal brain from Patient No. 44 and corpus callosum (Figure 5)." makes perfectly good sense. By "glial tumors" Applicants are referring to the GMBs and astrocytomas. Meningioma is a NON-MALIGNANT tumor so its lower level of expression is expected. As explained above corpus callosum is a valid control and the variation in expression of α4 laminin subunit gene from various normal brains as compared to corpus callosum is not surprising because the normal brain samples have variable amounts of neuronal tissue whereas corpus callosum is almost purely glial in tissue type. What is important is that as shown in Fig. 1A very few of the measured genes (including α4 the laminin subunit gene) in the comparison between corpus callosum and normal brain

fall beyond the ±2 lines whereas the expressions in malignant tissue do fall beyond these lines. The discussion of the various levels of gene expression are a product of the extremely quantitative nature of the measurements—in direct contradiction of the Examiner's contention that the experiments are not quantitative. It should also be pointed out that Table 7 deals with detection of actual protein (as opposed to nucleic acids) which detection is covered by the non-elected claims and, hence, Table 7 does not directly apply to the claims in prosecution.

To recapitulate, the inventive method teaches how to compare potentially abnormal samples to a control and determine if the level of α4 laminin subunit gene expression is sufficiently high as to indicate malignancy or abnormal cells on the way to malignancy (indication of recurrence). Gene expression above the 2 line in the histograms represents clearly abnormal expression. Therefore Applicants respectfully request the Examiner to withdraw her statement that "There is no indication in the specification of a threshold which could be indicative of malignant tumors, namely brain tumors. Therefore, distinguishing a malignant tumor tissue from a normal tissue based solely on different sample expression would be unpredictable."

These same discussions also apply to the Examiner's contentions concerning Claims 28-29, 32-36, 44-45, 48-59 directed to a method of predicting recurrence. Since the method provides means for determining the normal threshold for α4 laminin and other gene expression, it is relatively straightforward to apply these thresholds and conclude that the more α4 laminin subunit gene expression departs from this threshold in the tissue, the more likely a rapid recurrence. The presented data show a high degree of

correlation between the inventive analysis and clinical outcome, thereby negating the Examiner's contention that the outcome would be unpredictable.

In terms of Claims 60-66 the Examiner's attention is drawn to the discussions of actual laminin isotypes on pages 49 and later. Applicants have found that the expression of Laminin 8 ($\alpha 4\beta 1\gamma 1$) is uniquely correlated with high grade malignancies. Since the $\gamma 1$ subunit is widely expressed, actual expression of Laminin 8 requires an overexpression of both $\alpha 4$ and $\beta 1$ subunits (this being the other overexpressed structural gene). Given the quantitative underpinnings of the existing method, it is relatively simple to make the required measurements and arrange the samples in order of overexpression of the specified genes. This order then becomes the claimed grade. The exciting thing here is that the grade order is truly predictive of tumor behavior whereas tumor grades established on cytological and other characteristics are often not predictive of behavior. In the case of laminin 8 just discussed, GBMs have laminin 8 whereas most astrocytomas do not. Thus, the ranking would establish GBMs as the most aggressive, etc. Those few astrocytomas showing laminin 8 or other GBM-like overexpression would receive a rank close to GBM—something that would never occur with traditional cytological ranking.

In terms of the Examiner's statement on page 6 concerning the lack of applicability to other malignant tumors (beyond brain tumors) the Examiner is asked to consider the mention of these other tumors in the specification and the discussion of why Applicants believe that $\alpha 4$ laminin subunit overexpression correlates with malignancy. The role of angiogenesis in malignancy is well established. The relation between vascular tissue and $\alpha 4$ laminin suggest a mode by which this structural gene can be related to

malignancy. The inventive method sets out the roadmap that is readily applicable to other malignancies. Essentially, one determines the normal level of α4 laminin subunit gene expression in a tissue and then determines the level of α4 laminin subunit gene expression in various malignancies common to that tissue. The statements concerning using the method on a number of other tissues were made in the specification because preliminary tests were made prior to filing the application. These tests indicated that other malignancies also share the overexpression of α4 laminin subunit gene that defines malignant brain tumors. The Examiner is directed to the enclosed Declaration from one of the inventors, Dr. Ljubimova. The Declaration confirms that prior to the filing of the instant application preliminary experiments showed that breast cancer also exhibits α4 laminin overexpression. Since the filing of the application, a complete breast malignancy study has been completed. Dr. Ljubimova presents those results demonstrating that the methods of the instant invention are directly applicable to breast malignancy. Additional preliminary results also show that the method is applicable to malignancy of the prostate.

Thus far, each malignancy examined has demonstrated ready applicability of the inventive method. This method is the first method to employ α4 laminin with malignancy. Applicants have provided explicit directions for applying the method. It takes little undirected experimentation to confirm that a given malignancy shows the α4 laminin overexpression. While it is not yet known if every single malignancy will show the effect, enough is now known to indicate that any tissue showing α4 laminin elevated above normal is at least highly suspicious. Applicants respectfully contend that they have provided a method widely applicable to the detection of malignancy.

Therefore Applicants respectfully request the claim rejections under 35 U.S.C. 112, first paragraph be withdrawn.

Rejections Under 35 U.S.C 112, second paragraph.

The claims have been amended to correct the lack of antecedent basis pointed out by the Examiner. Applicants respectfully request that the claim rejections for lack of antecedent basis be withdrawn.

In terms of the "relatively highly invasiveness" and related terminology of Claims 60-66 Applicants point out that the terms of invasiveness and aggressiveness are well understood in the fields to which the current invention apply. An invasive tumor is one that readily metastasizes and/or quickly penetrates adjacent tissues. An aggressive tumor is one that expands and grows rapidly and/or resists conventional treatments. Either of these two characteristics renders a given malignancy more serious. A tumor having both features is of the gravest nature. The words "relatively highly" were an attempt to indicate the comparative significance of the factors. The idea is that the quantitative measurement of the factors directly translates into a degree of invasiveness or aggressiveness. For example, if two tumors both overexpressed *laminin α4*-specific mRNA (with respect to the control as well as overexpressing a particular structural gene and the first tumor had a laminin score of 30 times the control and a score of 10 times the control for the structural gene while the second tumor had a laminin score of 10 and a structural gene score of 8, the first tumor would be ranked as more invasive than the second tumor. Claim 60 has been amended to make this concept clearer. Applicants respectfully request the withdrawal of the rejections of Claims 60-66.

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application, as amended, are requested.

If for any reason the Examiner still finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles telephone number (310) 734-5200 to discuss the steps necessary for placing the application in condition for allowance.

You are hereby authorized to charge any fees due and refund any surplus fees to our Deposit Account No. 50-2567.

Respectfully submitted,

REED SMITH CROSBY HEAFEY

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Attachment: Declaration of Dr. Ljubimova